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Presence of *rfe* Genes in *Escherichia coli*: Their Participation in Biosynthesis of O Antigen and Enterobacterial Common Antigen

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In *Salmonella*, *ilv*-linked *rfe* genes participate in the biosynthesis of the enterobacterial common antigen (CA) as well as of certain types of O antigen (serogroups C_1 and L). *rff* genes, probably in the same cluster with *rfe*, are required for CA synthesis (P. H. Mäkelä et al., in preparation). Several *Escherichia coli* strains were studied to determine whether they also have *rfe-rff* genes that are involved in the synthesis of O antigen and CA, or of CA only. In a first approach, *E. coli* K-12 F-prime factors carrying the genes *ilv* and *argH* or *argE* and presumably *rfe-rff* genes were introduced into CA-negative *Salmonella* mutants that are blocked in CA synthesis because of mutated *rfe* or *rff* genes. All resulting ilv^+ hybrids were CA positive. In recipients with group C_1-derived *rfb* genes, the synthesis of O6,7-specific antigen was also restored. This result shows that *E. coli* K-12 has *rfe* and *rff* genes providing the functions required in the synthesis of CA and *Salmonella* 6,7-specific polysaccharide. By introduction of defective *rfe* regions from suitable *Salmonella* donors into *E. coli* O8, O9, and O100 strains, the synthesis of CA as well as of the O-specific polysaccharides was blocked. This indicates that in the *E. coli* strains tested the *rfe* genes are involved in the synthesis of both O antigen and CA. This suggestion was confirmed by the finding of *E. coli* rough mutants that had simultaneously become CA negative. In transduction experiments it could be shown that the appearance of the rough and CA^- phenotype was due to a defect in the *ilv*-linked *rfe* region.

The O-antigenic lipopolysaccharide (LPS) of *Enterobacteriaceae* consists of an O-specific polysaccharide linked to a core oligosaccharide, which in turn is bound via 2-keto-3-deoxyoctonate to lipid (11). It is known from genetic studies with *Salmonella* that the biosynthesis of the core and the O-specific polysaccharide chains is under control of *rfa* and *rfb* genes, respectively (27). Most of the *rfa* genes are clustered in the *cysE-pyrE* region, whereas the *rfb* genes form a cluster closely linked to the *his* operon.

Gene regions equivalent in site and function to the *Salmonella rfa* and *rfb* genes have been demonstrated in *Escherichia coli* also (17, 25).

Recently, Mäkelä et al. (13) found another gene cluster, called *rfe*, in *S. minnesota* (serogroup L) and *S. montevideo* (serogroup C_1). The *rfe* genes are located near the isoleucine and valine gene *ilv*. A mutation in this region blocks the synthesis of O-specific polysaccharides in these groups, and the resulting rough (R) mutants thus resemble phenotypically *rfb*-defective mutants. Moreover, it could be shown (14, 15) that *rfe* mutants are unable to synthesize the enterobacterial common antigen (CA). Therefore, it was suggested that *rfe* forms a cluster of genes partly required for O-chain synthesis in certain O groups and partly involved in CA synthesis.

S. typhimurium (serogroup B) also has *ilv*-linked *rfe* genes that can support the production of O6,7-specific polysaccharide, but they are not required for group B-specific (4, 12) O-chain synthesis (14). Moreover, CA synthesis in *S. typhimurium* seems to be determined by *ilv*-linked genes (*rfe* and/or *rff*; see below) and *rfb* genes, whereas in *S. montevideo* all genetic CA determinants so far recognized are assembled in the *ilv* region (14). This became evident when the introduction of the group C *rfb* from *S. montevideo* (serogroup C_1) into *S. typhimurium* led to smooth(S) (O6,7) hybrids that showed a dramatically reduced CA synthesis (CA detectable only in trace amounts; 14).

In *S. typhimurium* strains with an extended deletion of the *his-rfb* chromosomal region, *ilv*-

linked mutations have been identified that affect the synthesis of CA but not the ability to support the synthesis of O4,12- or O6,7-specific polysaccharides. The genes involved were provisionally called *rff* but may belong to the *rfe* gene cluster (P. H. Mäkelä, H. Mayer, G. Schmidt, H. Nikaido, H. Y. Whang, and E. Neter, manuscript in preparation).

Experiments described in this investigation demonstrate the presence of *ilv*-linked *rfe* genes in various *E. coli* serotypes. These genes, like those in *S. minnesota* and *S. montevideo*, are involved in the synthesis of both the O-specific polysaccharide and enterobacterial common antigen.

MATERIALS AND METHODS

Bacteria. The bacterial strains and their characteristics are listed in Table 1.

Conjugation. For conjugation experiments,

TABLE 1. *Bacterial strains used*

Strain	Species	Mating type	Relevant genotype[a]	Serotype	CA[b]	Reference or derivation
F440	*Escherichia coli*	Hfr40		O8:K27	+	25
AB1206	*E. coli* K-12	F14	*lacY1 thi⁻his⁻pro⁻*/F14 *ilv⁺ argH⁺*	R	+	18; received as CGSC 1206[c]
KLF33/JC1553	*E. coli* K-12	F133	*lac⁻arg⁻met⁻his⁻leu⁻*/ F133 *ilv⁺ argE⁺*	R	+	10; received as CGSC 4265
F464	*E. coli*	F⁻	*mtl⁻his⁻str^r*	O8:K27⁻	+	F492
F492	*E. coli*	F⁻		O8:K27⁻	+	24
F697	*E. coli*	F⁻	*ilv⁻his⁻*	O100	+	
F760	*E. coli*	F⁻	*ilv⁻rha⁻his⁻str^r*	O8:K27	+	*E. coli* 56b(O8:K27)
F1312	*E. coli*	F⁻	*ilv⁻rha⁻his⁻str^r*	O8:K27⁻	+	F760
EH703	*E. coli*	F⁻	*ilv⁻*	O9:K29⁻	+	
EH712	*E. coli*	F⁻	*ilv⁺ B-rfe-4274*	R	−	Recombinants from cross SH5454 × EH703
EH714	*E. coli*	F⁻	*ilv⁺ B-rfe-4274*	R	−	
EH724	*E. coli*	F⁻	*rfe⁻*	R	−	Mutants of F492
EH725	*E. coli*	F⁻	*rfe⁻*	R	−	
EH726	*E. coli*	F⁻	*rfe⁻*	R	−	
SH672	*Salmonella montevideo*	HfrH14		O6,7	+	12
SH3862	*S. montevideo*	HfrH14	*leu-1357 rfe-3853*	R	−	14; mutant of SH672
SH4159	*S. typhimurium* hybrid	F⁻	*B-ilvA401 B-rfe⁺ C-rfb⁺*	O6,7	tr	14
SH4210	*S. typhimurium* hybrid	F⁻	*B-ilvA401 rfe-3946 C-rfb⁺*	R	−	Mutant of SH4159
SH4212	*S. typhimurium* hybrid	F⁻	*B-ilvA401 B-rfe-3948 C-rfb⁺*	R	−	Mutant of SH4159
SH4472	*S. typhimurium*	F⁻	*ilv-1180 rff-4273 B-rfb⁺*	O4,12	−	*his⁺rfb⁺* recombinant of SH4480 (Mäkelä et al., in preparation)
SH4480	*S. typhimurium*	F⁻	*ilv-1180 rff-4273 his-658*	R	−	*his-rfb* deletion (16; Mäkelä et al., in preparation)
SH4773	*S. typhimurium* hybrid	F⁻	*C-ilv-1183 C-rfe-3853 B-rfb⁺*	O4,12	−	14
SH5149	*S. typhimurium*	F⁻	*ilv-1189 rff-4271 his-399*	R	−	*his-rfb* deletion (16; Mäkelä et al., in preparation)
SH5150	*S. typhimurium*	F⁻	*ilv-1190 rff-4270 his-809*	R	−	*his-rfb* deletion (16; Mäkelä et al., in preparation)
SH5454	*S. typhimurium* hybrid	HfrK1-2	*serA13 rfa-3058 rfe-4274 C-his⁺ C-rfb⁺*	R	−	Derived from SA464 (21)

[a] Standard genetic symbols (20, 28) except the use of prefix B or C to indicate, when relevant, the origin of certain loci from *Salmonella* group B or C. *E. coli* strains differ from *Salmonellae* by being naturally lactose fermenting (*lac⁺*) and indole producing (*tna⁺* for tryptophanase).

[b] Determined by hemagglutination: +, present; −, absent; tr, trace amount.

[c] CGSC, Coli Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

freshly grown broth cultures of donor and recipient cells were mixed in a ratio of 1:10. This mating mixture was incubated at 37°C for 1 to 3 h. Selection of recombinants was performed on Davis minimal agar (7) suitably supplemented when necessary. This selective agar contained lactose as sole carbohydrate source to prevent the growth of the *lac*⁻ *Salmonella* donors or streptomycin (1 mg/ml) to kill the *E. coli* donors. The recombination frequency in crosses between *Salmonella* and *E. coli* was very low (about one to five per 10^7 input donor cells).

Recombinant colonies were first transferred onto the same medium used for selection and then streaked out on complete nutrient agar, from which single colonies were isolated. The recombinants purified by repeated single-colony isolations were tested for their nutritional and LPS characteristics and the presence of CA.

Transduction. For transduction experiments, we used phage P1*kc*, a derivative of P1. Phage lysates were prepared on the respective donor strain, using the agar layer method described by Adams (1). For propagation of the P1*kc* phage and for transduction, the following medium was used: tryptose (Difco), 10 g; yeast extract (Difco), 5 g; NaCl, 8 g; glucose, 1 g; and 1,000 ml of distilled water. This medium was supplemented with calcium chloride to 5×10^{-3} M. The resulting phage lysates usually contained approximately 10^{10} plaque-forming units. For transduction overnight, broth cultures of the recipients were diluted 20-fold in broth with calcium chloride and incubated at 37°C in a shaking water bath for 4 h. Equal volumes of broth culture and phage lysate were mixed to give a multiplicity of nearly 1. After incubation at 37°C for 20 min, 0.1-ml samples were plated on appropriately supplemented selective agar and incubated for 48 h at 37°C.

Bacteriophages. Recombinants and transductants were characterized by their reaction pattern against the following phages: Felix-O1 (FO), 6SR, Ω8, and PCO1c4. Phage Ω8 specifically lyses cells having the *E. coli* O8 antigen (5), and phage PCO1c4 attacks cells producing the *Salmonella* 6,7 antigens of group C_1 (P. H. Mäkelä, manuscript in preparation). FO phage lyses S and R bacteria having the complete *Salmonella* LPS core (9) or the complete *E. coli* R2 core (24). The R-specific phage 6SR attacks R forms with LPS cores of different types (22). In addition, we used the "capsule phage" 27, which lyses cells carrying the *E. coli* K27 capsule (26). The phages were propagated on suitable hosts in broth (1) and stored over chloroform at 4°C. To determine the susceptibility of bacteria to phages, drops of lysates containing about 10^8 plaque-forming units per ml were deposited onto bacterial lawns on agar. The phage reaction was scored after overnight incubation at 37°C.

Indole formation. This was determined by the addition of Kovacs reagent to cultures grown overnight in 1% tryptose (Difco).

CA determination. The presence or absence of CA was determined by the passive hemagglutination test as described previously (15).

Serological methods. The LPS characteristics were determined by slide agglutination tests with appropriate O antisera suitably diluted in 0.2% saline to avoid spontaneous agglutination of R mutants and with 4% saline to discriminate between S and R forms.

Chemical methods. LPS was extracted by phenol-water and centrifuged at 105,000 × *g*. LPS was obtained as sediment, whereas the supernatant represents the L_1 fraction, which in certain cases may contain O-specific hapten (2). The analyses of these fractions were performed by gas-liquid chromatography as described previously (23).

RESULTS

Introduction of the *rfe* region of *E. coli* K-12 into *Salmonella*. In *Samonella* we have a test system for the function of *rfe* genes: synthesis of CA and synthesis of O antigen of specificity 6,7 (as determined by *rfb* genes of group C) or 21 (as determined by *rfb* genes of group L). We planned to test whether *E. coli* would have *rfe* genes that could perform these functions by introducing the chromosomal region close to *ilv* and presumably harboring the *rfe* equivalent from *E. coli* into suitable CA-negative *Salmonella* strains as indicators. As the number of recombinants obtained in such interspecies crosses is usually very low, it was important to have an efficient donor strain of *E. coli*. The F-prime factors F14 and F133 of the *E. coli* donors AB1206 and KLF33/JC1553, respectively, looked very promising: since they were known to carry the genes *ilv* and *argH* or *argE*, respectively, it was likely that they would also carry *rfe* and *rff* alleles.

Table 2 shows the results of crosses in which the strains containing F14 or F133 were used as donors; the recipients were various CA-negative *Salmonella* derivatives, and the mating took place on minimal glucose medium (containing histidine) for the selection of *ilv*⁺ recombinants. Such recombinants were tested for CA and LPS characteristics. All recombinants were CA positive, indicating that the K-12 *rfe* region (presumably also comprising *rff* genes) was capable of supporting CA synthesis irrespective of the status of the *rfb* genes. The K-12 *rfe* is in this respect similar to *Salmonella* group C *rfe* and more complete than the group B *rfe* (14).

The recombinants of crosses involving recipients with group C-derived *rfb* genes were also smooth and agglutinated in the anti-6,7 serum (Table 2). Therefore the K-12 *rfe* genes can be deduced to provide the *rfe* function required in the synthesis of the 6,7-type O antigen; in this respect it is like C *rfe* or B *rfe*. It is possible that the K-12 *rfe* does not restore the capacity to synthesize the 6,7-type polysaccharide to maximal efficacy—some of the *ilv*⁺ recombinants

TABLE 2. *CA and LPS characteristics of ilv⁺ recombinants that received the F14 or the F133 episome from E. coli*[a]

Designation	Recipient strain					*ilv*⁺ hybrids that received given episome			
						F14		F133	
	rfe[b]	*rff*	*rfb*	CA	LPS	CA	LPS	CA	LPS
SH4773	C−		B+	−	4,12	+	4,12	No recombinants	
SH4159	B+	(+)	C+	tr[c]	6,7	+	6,7[d]	+	6,7[d]
SH4210 from SH4159	B−		C+	−	R	+	6,7[d]	+	6,7[d]
SH4212 from SH4159						+	6,7[d]	No recombinants	
SH5150 from *his-809*						No recombinants		+	R
SH5149 from *his-399*	B+	(−)	B_{del}[e]	−	R	+	R	+	R
SH4480 from *his-658*						+	R	+	R
SH4472 from SH4480	B+	(−)	B+	−	4,12	+	4,12	+	4,12

[a] Recipients were *ilv*⁻ derivatives of *S. typhimurium* LT2.

[b] The prefix C or B denotes origin of these genes from either group C or group B *Salmonella*. In parentheses is the status of the *rfe*-related *rff* gene(s) (Mäkelä et al., in preparation) when known.

[c] tr, Trace amount detectable by hemagglutination (14; Mäkelä et al., in preparation).

[d] These recombinants were smooth and 6,7 as judged by their agglutination reactions, but partly sensitive to some R-specific phages.

[e] B_{del}, Extended deletion in the *his-rfb* chromosomal regions (16).

were sensitive to R-specific phages even when agglutinating specifically with the anti-6,7 serum. Previous experience with leaky R mutants (7) has shown that this is the behavior seen when O-antigen synthesis proceeds at less than maximal capacity: some R-core stubs remain uncapped by the O side chains and serve as receptor sites for the R-specific phages. This observation does not change the conclusion that K-12 *rfe* genes have the function required for the synthesis of the 6,7-type polysaccharide—the *ilv*⁺ recombinants were definitely different from their R-recipient parent in possessing 6,7 specificity.

Introduction of a defective *Salmonella rfe* region into *E. coli*. The experiments described above show that the allelic region of *E. coli* equivalent to the *Salmonella rfe* genes can replace missing functions of *rfe*-defective mutants of *Salmonella*. They do not tell, however, whether *rfe* genes are also required in *E. coli* for the synthesis of either O antigen or CA. Therefore we performed conjugation experiments with the aim of introducing the defective *rfe* region of a *Salmonella* donor into various *E. coli* serotypes by selection of the donor *ilv*⁺ genes. Many of the *ilv*⁺ hybrids were expected to be CA negative and rough, blocked in O-specific polysaccharide synthesis, if the *rfe* equivalent of *E. coli* is involved in the synthesis of both these antigens.

As donor we used *S. montevideo* SH3862, an *rfe*⁻ mutant of HrfH14 (12). In a first mating, the recipient was the multiply marked *E. coli* F760 with the O antigen 8 and the capsular (K) antigen 27. On suitably supplemented minimal agar, *ilv*⁺*lac*⁺ recombinants were selected. A total of 13 hybrids were obtained. After single-colony isolation they were tested for unselected markers, for presence of CA, and for their LPS characteristics (Table 3). The genetic markers relevant for this investigation are arranged in Table 3 according to their assumed order on the chromosomal linkage map (20, 28). All 13 hybrids had retained the *his*⁻ marker. Three of the hybrids (classes e, f, and g) were unchanged in their antigenic patterns and produced CA. The remaining 10 *ilv*⁺ hybrids were CA negative and did not synthesize the O8 antigen (Ω8 resistant). Three of these 10 hybrids (class a) were sensitive to phage FO, indicating that they presumably synthesize a *Salmonella* LPS core or a *Salmonella*-like core due to the transfer of *Salmonella rfa* genes. All the CA⁺ as well as CA⁻ hybrids still possessed the capsular antigen K27, as shown by their sensitivity to phage 27 and by their agglutinability in anti-K27 serum. Due to the presence of the capsule, the hybrids showed typical traits of S forms; e.g., they were nonagglutinable in 4% saline and formed homogeneously turbid suspensions even after heating (1 h, 100°C). The sensitivity of the CA⁻ recombinants to the R-specific phage 6SR together with their resistance to phage Ω8 indicates that they contain R-type LPS.

It is known that in *S. minnesota* and *S. montevideo rfe* defects block O-specific hapten synthesis. Therefore, some of the O8⁻, CA⁻ hy-

brids were investigated for the presence of O8-specific hapten. No O-specific hapten could be found.

These results suggest that the missing biosynthetic functions in the production of O8 antigen and CA in the *ilv*$^+$ hybrids have been caused by the introduction of the defective *Salmonella rfe* region, which has replaced respective intact *E. coli* genes. This could be checked again by the reintroduction of the *E. coli* chromosomal segment assumed to contain the *rfe* genes. Therefore, one hybrid group (class d of Table 3) was remated with the *E. coli* donor Hfr40 (O8), which transfers its chromosome clockwise with *xyl* as leading marker (25). The selective minimal agar contained rhamnose as sole carbon source and streptomycin for counterselection of the donor. Twenty *rha*$^+$ clones were isolated and tested for the presence of CA and O8 antigen. The outcome of the tests showed that all hybrids had retained the *his*$^-$ recipient allele. One of the hybrids was still O8$^-$ as well as CA$^-$ and did not produce indole (*tna*$^-$). The remaining 19 hybrids, however, synthesized the O8 antigen and CA and were indole positive (*tna*$^+$). Thus the incorporation of the donor chromosomal segment comprising the *tna-rha* genes could restore the CA and O8 antigen synthesis, presumably in consequence of the introduction of intact *E. coli rfe* genes.

Introduction of *S. montevideo rfb* (C *rfb*) into *E. coli* O8. As mentioned above, the CA synthesis of *S. typhimurium* is under joint control of *rfb* genes and *ilv*-linked *rfe* and/or *rff* genes. In *S. montevideo* (14) and *E. coli* K-12 (see Table 2), all genetic CA determinants seemed to be clustered in an *ilv*-linked region, whereas *rfb* genes do not have any role in CA production (14). To test whether in *E. coli* O8 also the *rfe* gene cluster is alone capable of supporting the synthesis of CA, we crossed the *S. montevideo* (O6,7) donor SH672 with *E. coli* F464 (O8), selecting recombinants with the intact donor *his* region.

Seventeen such *his*$^+$ clones were isolated and tested for unselected markers and O antigens (Table 4). Nine of the *his*$^+$ hybrids were found to have the *Salmonella* O6,7 specificity in con-

TABLE 3. *Inheritance of unselected markers and phage sensitivity of 13 ilv*$^+$ *hybrids from a cross of the S. montevideo donor SH3862 (rfe*$^-$*) with E. coli F760 (O8:K27)*[a]

No. of hybrids	Class[b]	Gene marker					Production of:		Phage sensitivity[c]			
		rfa[d]	*tna*	*ilv*	*rfe*	*rha*	CA	Indole	Ω8	27	FO	6SR
3	a	1[e]	1	1	1	0	−	−	−	+	+	+
3	b	0	1	1	1	1	−	−	−	+	−	+
3	c	0	0	1	1	1	−	+	−	+	−	+
1	d	0	1	1	1	0	−	−	−	+	−	+
1	e	0	1	1	0	1	+	−	+	+	−	−
1	f	0	1	1	0	0	+	−	+	+	−	−
1	g	0	0	1	0	0	+	+	+	+	−	−
Proportion of donor characters/total		3/13	9/13	13/13	10/13	7/13						
Linkage		0,23	0,69	1	0,77	0,62						

[a] Donor phenotype (SH3862): Tna$^-$, Ilv$^+$, Rha$^+$, CA$^-$(*rfe*$^-$), Fo^s, Ω8^r, ϕ27^r, 6SR^s; recipient phenotype (F760): Tna$^+$, Ilv$^-$, Rha$^-$, CA$^+$ (*rfe*$^+$), Fo^r, Ω8^s, ϕ27^s, 6SR^r.

[b] According to gene constitution.

[c] +, Lysis; −, no reaction.

[d] Donor *rfa* genes indicated by sensitivity to FO, which has its receptor in the *Salmonella* LPS core (9).

[e] Donor character indicated by 1. Recipient character indicated by 0.

TABLE 4. *Analysis of 17 his*$^+$ *hybrids from a cross between S. montevideo HfrH14 and E. coli F464 (O8,*

No. of hybrids	Phage sensitivity[a]				CA[b]	Reaction in anti-O6,7 serum	Carbohydrate utilization	
	Ω8	$PCO1_{c4}$	FO	6SR			Lactose	Mannitol
9	−	+	−	−	+	+	+	−
8	+	−	−	−	+	−	+	−
Parents								
E. coli F464	+	−	−	−	+	−	+	−
HfrH14	−	+	+	−	+	+	−	+

[a] +, Lysis; −, no reaction.

[b] +, Presence of CA.

sequence of the introduction of the *his*-linked donor *rfb* region. The CA activity of the O6,7 hybrids to sensitize erythrocytes for passive hemagglutination was as strong as that of the parent strains and the O8 hybrids. Thus 0.01-ml volumes of a saline extract of the hybrids could sensitize the erythrocytes to be agglutinated to maximal titers of a potent CA antiserum. The introduction of the *his*-linked C *rfb* into *S. typhimurium* led to O6,7 hybrids synthesizing only trace amounts of CA that were detectable by using at least 1 ml of saline extract (compared to 0.01 ml used with wild [CA^+] types) for erythrocyte sensitization (15).

This demonstrates that, as shown above in *Salmonella* strains of groups C_1 and L and *E. coli* K-12, in *E. coli* O8 also the intact *rfe* region suffices for CA synthesis irrespective of the *rfb* region.

Isolation of *rfe*-defective mutants from *E. coli* O8. If *E. coli* O8 behaves, as suggested above, like *Salmonella* of group C_1 requiring the *rfe* function for the synthesis of both the CA and the O-specific polysaccharide, it ought to be possible to find *rfe*$^-$ mutants of *E. coli* O8 from among R mutants (which are easy to isolate). We therefore looked for R mutants as rough-looking colonies after treating with diethyl sulfate (13) a broth culture of a K^- mutant (F492) of *E. coli* O8:K27. Three colonies (from independent experiments: EH724 through EH726) agglutinating in 4% NaCl and sensitive to the R-specific phage 6SR were CA negative. It seemed that in these a mutation had occurred that, like *rfe* mutations, simultaneously prevented the synthesis of CA and of the O8-specific polymer. The smooth (O8) and CA^+ phenotype could be restored by introducing the F133 episome (in crosses like those in Table 2) from the *E. coli* K-12 donor and by selecting for phage 6SR-resistant recombinants.

To determine the mutation site leading to the rough, CA^- phenotype more precisely, we performed P1 transductions, using *E. coli* EH726 (CA^-, R) as donor and the *E. coli* O8 strain F1312 (CA^+, *ilv*$^-$) as recipient. One hundred *ilv*$^+$ transductants were selected on appropriate selective medium and tested for their LPS characteristics and presence of CA. The results (Table 5) show that 81% of the *ilv*$^+$ transductants had both the R and CA^- phenotypes of the donor due to defective donor alleles that are cotransducible with *ilv*. From the results of conjugation and transduction experiments, we conclude that the mutations in the three R mutants, EH724 through EH726, of *E. coli* O8 had occurred in the *ilv*-linked *rfe* genes.

Introduction of defective *Salmonella rfe* regions into *E. coli* O9 and O100. In a cross similar to that described before, a K^- mutant (EH703) of *E. coli* O9:K29 (*ilv*$^-$) as recipient was crossed with the donor SH5454, an *rfe*$^-$ mutant of *S. typhimurium* HfrK1-2. Twenty-three *ilv*$^+$ recombinants were isolated; they were all lactose fermenting like the *E. coli* recipient. Of these 23, 16 were CA^+ and smooth (O9) like the recipient, whereas 7 were CA^- and rough, as indicated by their sensitivity to the R-specific phage 6SR and by their agglutinability in 4% saline. From two of the CA^- recombinants (EH712 and EH714) LPS and L_1 fractions (O-specific hapten) were isolated and shown to be devoid of mannose, the main constituent of the O9-specific polymer (6).

TABLE 5. *CA and LPS characteristics of ilv*$^+$ *transductants from P1 transduction with EH726 (CA*$^-$*, R) as donor and E. coli O8 F1312 (CA*$^+$*, ilv*$^-$*) as recipient*

No.	Phage reaction[a]		LPS[b]	CA[c]	Unselected markers	
	Ω8	6SR			*rha*	*his*
81	−	+	R	−	−	−
19	+	−	S	+	−	−
Parents						
EH726	−	+	R	−	+	+
F1312	+	−	S	+	−	−

[a] +, Lysis; −, no reaction.
[b] R, Rough-type LPS; S, smooth O8-specific LPS.
[c] +, CA present; −, CA absent.

In another experiment we used the *E. coli* O100 strain F697 (*ilv*$^-$, *his*$^-$) as recipient in a cross with the *S. montevideo* Hfr donor SH3862 (*rfe*$^-$). Selection was made again for the donor *ilv* genes on suitable selective medium. Twenty-one *ilv*$^+$ hybrids were isolated and tested for unselected markers and LPS characteristics. All hybrids were lactose fermenting. Twelve of the hybrids were CA negative and rough as evidenced by their agglutinability in 4% saline, sensitivity to the R-specific phage 6SR, and nonreactivity in anti-O100 serum. The remaining nine recombinants were smooth (O100) and produced CA.

The simultaneous appearance of the CA-negative and R characters in the *ilv*$^+$ hybrids derived from *E. coli* O9 and O100 suggests that the *rfe*$^-$ of the respective *Salmonella* donor had replaced the *rfe*$^+$ genes of the recipients. The lack of the *rfe* functions obviously prevented the synthesis of both CA and O-specific polysaccharides. This suggests that in *E. coli* O9 as well as O100, *ilv*-linked *rfe* genes are involved in the synthesis of both O antigen and CA.

DISCUSSION

The data presented here demonstrate that in various *E. coli* serotypes (O8, O9, O100), as in

S. montevideo and *S. minnesota*, *ilv*-linked *rfe* genes participate in CA as well as O-antigen synthesis. It is noteworthy, however, that *rfe* genes do not seem to be involved in the synthesis of the capsular antigen K27 (see Table 3).

With *E. coli* lines K-12 and O8, it could be shown that all the genes necessary for CA synthesis are located in the *rfe* cluster, as is the case in *Salmonellae* of serogroups C_1 and L (14, 15). This is in contrast to *S. typhimurium* (serogroup B), in which the genes involved in CA production are distributed among the *ilv*-linked *rfe-rff* and the *his*-linked *rfb* clusters (14). Thus different systems exist among enteric bacteria with regard to the localization of genes participating in CA synthesis. That *Salmonellae* of group B do not require functional *rfe* genes for their O-antigen synthesis seems to be another feature distinguishing these bacteria from several serogroups of *E. coli* and *Salmonellae* of groups C_1 and L.

Recently, glyceraldehyde phosphate was found at the reducing terminus of the haptenic O repeat units of *S. montevideo* (group C_1) (3). This compound was also detected in *E. coli* O8 at the reducing end of its O-specific hapten (3). The group B-specific (4, 12) hapten, the synthesis of which is independent of *rfe* genes, does not contain glyceraldehyde (3). It is therefore possible that the *rfe* genes of *S. montevideo* and *E. coli* O8 are involved in the biosynthesis of the intermediary hapten product containing glyceraldehyde phosphate.

The different steps of O-antigen synthesis in *Salmonellae* of groups B and E have been well established (19). Thus the repeat units are synthesized on a lipid carrier, called ACL (antigen carrier lipid), which is a C_{55} isoprenol. The polymerization of the O repeating units to a linear polymer still linked to the ACL is directed by a gene(s), designated *rfc*, which in group B maps far from the *rfb* (27). Recent experiments with *E. coli* strains led to the suggestion that a polyprenol-type lipid is not involved in the *E. coli* O9 antigen synthesis (6) and presumably also not in the synthesis of the structurally related O8 antigen (K. Jann, personal communication). Similar observations were made by J. Gmeiner (personal communication) with regard to the O-antigen synthesis in *S. montevideo*.

A gene locus equivalent in site or function to the *rfc* in *Salmonella* strains of group B could be detected neither in *Salmonella* strains of groups C_1 and L nor in *E. coli* O8 and O9, which in turn need intact *rfe* genes for their O-antigen synthesis. Thus the data accumulated in earlier studies with *Salmonella* (11-13, 15) and *E. coli* (6) and in this work with *E. coli* suggest that different mechanisms for the biosynthesis of O-antigenic polysaccharides may exist among the *Enterobacteriaceae*.

The precise role of the *rfe* genes and their products in the synthesis of certain types of O antigen as well as of the enterobacterial common antigen remains to be elucidated.

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